Claims

1. A recombinant DNA molecule comprising:
   (a) at least one first regulatory sequence of an intron of the Vascular Endothelial Growth factor (VEGF) receptor-2 (Flk-1) gene or of an intron of a gene homologous to the Flk-1 gene being capable of conferring expression in endothelial cells in vivo; and
   (b) operatively linked thereto a heterologous DNA sequence.

2. The recombinant DNA molecule of claim 1, wherein said first regulatory sequence comprises a GATA-binding site, an AP-1 binding site, an SP1 binding site, an NFκB binding site, a STAT binding site, a Scl/Tal-1 binding site, an Ets-1 binding site, a PEA3 consensus sequence or any combination(s) thereof.

3. The recombinant DNA molecule of claim 1 or 2, wherein said first regulatory sequence is selected from the group consisting of
   (a) DNA sequences comprising a nucleotide sequence as given in SEQ ID NO: 1;
   (b) DNA sequences comprising a nucleotide sequence of SEQ ID NO: 1 from nucleotide 8260 to nucleotide 10560, from nucleotide 8336 to nucleotide 10608 and/or from nucleotide 10094 to nucleotide 10608;
   (c) DNA sequences comprising the nucleotide sequence of the human Flk-1-intron;
   (d) DNA sequences comprising a nucleotide sequence which hybridizes with a nucleotide sequence of (a), (b) or (c) under stringent conditions;
   (e) DNA sequences comprising a nucleotide sequence which is conserved in the nucleotide sequences of (a), (b) and (c); and
   (f) DNA sequences comprising a fragment, analogue or derivative of a nucleotide sequence of any one of (a) to (e) capable of conferring expression in endothelial cells.

AMENDED SHEET

4. The recombinant DNA molecule of ~~any one of claims 1 to 3,~~ wherein said heterologous DNA sequence is operatively linked to further regulatory sequences.

5. The recombinant DNA molecule of claim 4, wherein said further regulatory sequence is a promoter.

6. The recombinant DNA molecule of claim 4 ~~or 5~~, wherein said further regulatory sequence is a 3'-untranslated region.

7. The recombinant DNA molecule of claim 5 ~~or 6~~, wherein said promoter is a promoter of hypoxia inducible genes, genes encoding growth factors or its receptors or glycolytic enzymes.

8. The recombinant DNA molecule of claim 7, wherein said growth factor is VEGF, PDGF or Fibroblast growth factor.

9. The recombinant DNA molecule of any one of claims 5 ~~to 8~~, wherein said promoter comprises a DNA sequence selected from the group consisting of
   (a) DNA sequences comprising the nucleotide sequence as given in SEQ ID NO:1 from nucleotide 6036 to nucleotide 6959;
   (b) DNA sequences comprising the nucleotide sequence of the human Flk-1/KDR promoter;
   (c) DNA sequences comprising a nucleotide sequence which hybridizes with a nucleotide sequence of (a) or (b) under stringent conditions;
   (d) DNA sequences comprising a nucleotide sequence which is conserved in the nucleotide sequences of (a) and (b); and
   (e) DNA sequences comprising a fragment, analogue or derivative of a nucleotide sequence of any one of (a) to (d).

10. The recombinant DNA molecule of any one of claims 1 ~~to 9~~, wherein at least one of said DNA sequences is of human or murine origin.

11. The recombinant DNA molecule of any one of claims 1 ~~to 10~~, wherein said heterologous DNA sequence being operatively linked to said regulatory sequences is located 5' to said first regulatory sequence.

12. The recombinant DNA molecule of any one of claims 1 ~~to 11~~, wherein said heterologous DNA sequence encodes a peptide, protein, antisense RNA, sense RNA and/or ribozyme.

13. The recombinant DNA molecule of claim 12, wherein said protein is selected from the group consisting of Vascular Endothelial Growth Factor (VEGF), Hypoxia Inducible Factors 7(HIF), HIF-Related Factor (HRF), tissue plasminogen activator, p21 cell cycle inhibitor, nitric oxide synthase, interferon-γ, atrial natriuretic polypeptide and monocyte chemotactic proteins.

14. The recombinant DNA molecule of claim 12, wherein said protein is a scorable marker, preferably luciferase, green fluorescent protein or lacZ.

15. The recombinant DNA molecule of claim 12, wherein said antisense RNA or said ribozyme are directed against a gene involved in vasculogenesis and/or angiogenesis and/or tumors of endothelial origin.

16. A nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with the first regulatory sequence of a recombinant DNA molecule of any one of claims 1 ~~to 15~~.

17. A vector comprising a recombinant DNA molecule of any one of claims 1 ~~to 15~~.

18. The vector of claim 17, which is an expression vector and/or a targeting vector.

19. The vector of claim 17 ~~or 18~~, further comprising a gene capable of expressing HIF-2α.

20. A cell transformed with a DNA molecule of any one of claims 1 ~~to 15~~ or the vector of any one of claims 17 ~~to 19~~.

21. The cell of claim 20, which is a prokaryotic or eukaryotic cell.

22. The cell of claim 20 ~~or 21~~, which is an endothelial cell.

23. The cell of any one of claims 20 ~~to 22~~, further comprising a recombinant DNA molecule or vector containing a gene capable of expressing HIF-2α.

24. A pharmaceutical composition comprising a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~ and/or the nucleic acid molecule of claim 16 and optionally a pharmaceutically acceptable carrier.

25. A diagnostic composition comprising a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~, the cell of any one of claims 20 ~~to 23~~ and/or the nucleic acid molecule of claim 16, and optionally suitable means for detection.

26. A method for the production of a transgenic non-human animal, comprising introduction of a recombinant DNA molecule of any one of claims 1 ~~to 15~~ or a vector of any one of claims 17 ~~to 19~~ into a germ cell, an embryonic cell or an egg cell or a cell derived therefrom.

27. A transgenic non-human animal comprising stably integrated into its genome a recombinant DNA molecule of any one of claims 1 ~~to 15~~ and/or the vector of any one of claims 17 ~~to 19~~ or obtained according to the method of claim 26.

28. The method of claim 26 ~~or the transgenic non-human animal of claim 27~~, wherein said animal is a mouse.

29. A method for the identification of a chemical and/or biological substance capable of suppressing the transcription of a gene in endothelial cells comprising:

(a) contacting a cell of any one of claims 20 ~~to 23~~ or the transgenic non-human animal of claim 27 ~~or 28~~ either of which is capable of expressing the heterologous DNA sequence with a plurality of compounds; and

(b) determining those compounds which suppress the expression of said heterologous DNA sequence.

30. A method for the identification of a chemical and/or biological substance capable of activating and/or enhancing the transcription of a gene in endothelial cells comprising:

(a) contacting a cell of any one of claims 20 ~~to 23~~ or the transgenic non-human animal of claim 27 ~~or 28~~ either of which is capable of expressing the heterologous DNA sequence with a plurality of compounds; and

(b) determining those compounds which are capable of activating and/or enhancing the expression of said heterologous DNA sequence.

31. Use of a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~, the cell of any one of claims 20 ~~to 28~~, the pharmaceutical composition of claim 24, the diagnostic composition of claim 25 and/or the transgenic non-human animal of claim 27 ~~or 28~~ for the identification of a chemical and/or biological substance capable of suppressing or activating and/or enhancing the transcription, expression and/or activity of genes and/or its expression products in endothelial cells.

32. The method of claim 29 ~~or 30 or the use of claim 31~~, wherein the chemical and/or biological substance is selected from the group consisting of peptides, proteins, nucleic acids, antibodies, small organic compounds, hormones, neurotransmitters, peptidomimics and PNAs.

33. A method for the production of a pharmaceutical composition comprising the steps of the method of claim 29 ~~or 30~~ and (c) formulating the compound identified in step (b) in a pharmaceutically acceptable form.

34. A method of inhibiting a vascular disease in a subject, comprising contacting an artery of said mammal with the vector of any one of claims 17 ~~to 19~~, wherein

said heterologous DNA sequence encodes a protein that reduces or prevents the development of the vascular disease.

35. The method of claim 34, wherein said protein reduces proliferation of smooth muscle cells.

36. Use of a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~, the nucleic acid molecule of claim 16 and/or a substance identified by the method of claims 29, ~~30 or 32~~ for the preparation of a composition for directing or preventing expression of genes specifically in endothelial cells.

37. Use of a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~, the nucleic acid molecule of claim 16 and/or a substance identified by the method of claims 29, ~~30 or 32~~ for the preparation of a pharmaceutical composition for treating, preventing and/or delaying a vascular disease and/or a tumorous disease in a subject.

38. Use of a recombinant DNA molecule of any one of claims 1 ~~to 15~~, the vector of any one of claims 17 ~~to 19~~ and/or the nucleic acid molecule of claim 16 for the preparation of a pharmaceutical composition for inducing a vascular disease in a non-human animal or in the transgenic non-human animal of claim 27 ~~or 28~~.

39. The method of claim 34 ~~or 35~~ or the use of any one of claims 36 ~~to 38~~, wherein the vascular disease is atherosclerosis and/or a neuronal disorder.

40. Use of a regulatory sequence as defined in any one of claims 1 to 3 for enhancing and/or directing gene expression in endothelial cells.

add A1

add C9

IN THE CLAIMS:

Please amend the claims as follows:

In claim 4, line 1, please delete "any one of claims 1 to 3" and substitute therefor --claim 1--.

In claim 6, line 1, please delete "or 5".

In claim 7, line 1, please delete "or 6".

In claim 9, line 1, please delete "or 8".

In claim 10, line 1, please delete "to 9".

In claim 11, line 1, please delete "to 10".

In claim 12, line 1, please delete "to 11".

In claim 16, line 3, please delete "to 15".

In claim 17, line 1, please delete "to 15".

In claim 19, line 1, please delete "or 18".

In claim 20, please delete "to 15" and "to 19".

In claim 22, please delete "or 21".

In claim 23, please delete "to 22".

In claim 24, please delete "to 15" and "to 19".

In claim 25, please delete "to 15", "to 19" and "to 23".

In claim 26, please delete "to 15" and "to 19".

In claim 27, please delete "to 15" and "to 19".

In claim 28, please delete "or the transgenic non-human animal of claim 27".

In claim 29, line 4, please delete "to 23" and in line 5, please delete "or 28".

In claim 30, line 4, please delete "to 23" and in line 5, please delete "or 28".

In claim 31, line 1, please delete "to 15"; in line 2, please delete "to 19" and "to 23" and in line 4, please delete "or 28".

In claim 32, line 1, please delete "or 30 or the use of claim 31".

In claim 33, line 2, please delete "or 30".

In claim 34, line 2, please delete "to 19".

In claim 36, line 1, please delete "to 15"; line 2, please delete "to 19" and in line 3, please delete "30 or 32".

In claim 37, line 1, please delete "to 15"; line 2, please delete "to 19" and in line 3, please delete "30 or 32".